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14. ABSTRACT:
We aim to develop protein therapeutics that neutralize growth factors that activate EGF receptor family members in breast cancer. Rather than targeting receptors themselves (as do Herceptin, Iressa, etc), we propose to target the activating ligands. Our model is Argos from Drosophila, which we showed naturally inhibits EGF receptor signaling in fruit flies by inactivating the ligand. We hope to effectively 'humanize' Argos - making it bind human EGFR ligands and/or to use human protein scaffolds for this. In the past year, we crystallized a complex between the minimal functional fragment of Argos and its target (Spitz), and are about to complete structure determination – which will provide critical information for therapeutic design. We also established an experimental approach for screening libraries of Argos variants for those that bind human EGF-like ligands (our therapeutic aim). This approach employs yeast surface (rather than phage) display. We are now poised to combine our technical position and new structural information to identify Argos (and Dkk) variants that bind human EGFs and represent starting points for developing new therapeutics.

15. SUBJECT TERMS
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INTRODUCTION

The aim of this research project is to develop novel inhibitors of signaling through receptors from the epidermal growth factor (EGF) receptor family – well known to be important in breast cancer – by targeting (and neutralizing) the growth factor ligands rather than by targeting the receptors themselves. Apart from the relative success of Herceptin®, approaches that target ErbB receptors have yielded disappointing results in clinical trials, and this can be rationalized in retrospect. As described in a *Nature* letter [1] that we published in late 2004, we discovered that a physiologically important inhibitor of EGF receptor signaling in *Drosophila melanogaster* (named Argos) functions as a specific 'ligand-sink', neutralizing the fly's EGFR ligands and thus blocking signaling through this system. We propose to develop a detailed understanding of how Argos achieves this 'ligand-sink' activity, and to analyze possible orthologs of these inhibitory molecules in humans and other organisms. Our ultimate goal is to modify the growth factor-neutralizing abilities of human orthologs of Argos (or structurally related proteins), and provide the essential groundwork for developing an innovative approach for breast cancer treatment that will attack the ligands that activate EGF receptor family members rather than the receptors (which are the targets of all current therapies in this system). One of the central problems is that each ligand activates multiple receptors in the family: through a combination of direct and indirect interactions. In cancers caused by aberrant ligand-induced ErbB receptor signaling, an effective therapeutic strategy would require targeting of all four receptors simultaneously (for which no agents exist), or – more straightforwardly – one would target the activating ligand(s). Our approach should lead to a novel (but physiologically validated – in *Drosophila*) approach for achieving this.

Receptor tyrosine kinases (RTKs) from the EGF receptor family are well-validated therapeutic targets in breast and other cancers. The success of the ErbB2/HER2-targeted Herceptin® antibody [2] and more recently the ErbB2/EGFR tyrosine kinase inhibitor lapatinib [3] in breast cancer treatment has spurred efforts to achieve similar results with agents targeted to other receptors in this family. ErbB2/HER2/Neu is overexpressed to high levels in approximately 30% of breast cancer cases [4]. The EGF receptor is reported to be overexpressed in 14% - 91% of breast cancer patients [5]. It was therefore anticipated that therapeutic agents targeting the EGF receptor might show similar efficacy to Herceptin® in breast and other cancers [6]. However, although they looked very promising in preclinical studies [7, 8], EGF receptor inhibitors such as the cetuximab/Erbitux® antibody and tyrosine kinase inhibitors such as Iressa® and Tarceva® have yielded rather disappointing results in clinical trials [9-11], raising questions about whether the EGF receptor itself is really a good therapeutic target.

One of the key differences between ErbB2/HER2/Neu and the EGF receptor is that ErbB2 can be activated simply by over expression [12] (as occurs in breast cancer), whereas *EGF receptor still needs activating ligand even when over-expressed* [13]. It follows from this, and from the biology of ErbB receptors, that for clinical responses similar to those with Herceptin®, we should target the ligands of other receptors in the family rather than the receptors themselves. Developing a novel set of agents with this capability – based on our discovery that Drosophila use Argos to control their EGF receptor signaling in this way – is the aim of our current research.

BODY OF PROGRESS REPORT

In our original application, we proposed three central strategies for identifying or generating Argos-like molecules that will function as 'ligand-sinks' for the many growth factors that activate human EGF receptor family members:

- analyzing the ability of distant Argos homologs in humans to bind human EGF-related ligands
- adaptation of human proteins that are structurally related to Argos for action as ligand sinks in signaling by the human EGF receptor family
- adaptation of *Drosophila* Argos to bind (and neutralize) human EGF-related ligands

Our conviction is that this combination of strategies, critically supported by structural work on Argos and the Argos/ligand complexes plus *in vitro* biophysical and cell biological studies, will provide starting points for developing drugs that can sequester the growth factors that activate EGF receptor family members in cancer (rather than targeting the receptors themselves). We believe that such agents will have significant advantages over other drugs that are currently in (disappointing) trials for EGF receptor inhibition in breast and other cancers.

Task 1.
To test the hypothesis that hDkks bind the EGF domains of EGF-related growth factors, and can act as inhibitory ligand 'sinks' for signaling by ErbB receptors

As described in the 2006 progress report, we were successful in generating good quality recombinant hDkk1 and hDkk3 protein, but failed to detect any interactions between these proteins and human ErbB ligands. This negative finding suggests that our simplest route to identifying human Argos orthologs will not be productive. The likelihood of a positive result in these studies was remote at best, and – as discussed in the 2006 report, these negative findings have focused our attention more squarely on Aim 2 of the proposed research – to use both hDkk proteins and Argos as 'scaffolds' for developing 'anti-EGF' proteins that will bind and neutralize human ErbB ligands. To achieve this, we have focused on (and progressed with): (*i*) structural studies of Argos and Argos/Spitz complexes and (*ii*) developing methods to screen Argos (and hDkk) libraries for variants that will bind human ErbB ligands with high affinity.

Tasks 1a to 1c are all complete – although with negative results from the point of view of hDkk/ErbB ligand binding.

Task 1d *Perform additional biophysical and structural characterization of any positive interactions between hDkk proteins and human ErbB ligands, using analytical ultracentrifugation and X-ray crystallography (months 8 to 24)*

Task 1d remains active, but slightly refocused. Our rationale is as follows:
Based on the sequence analyses presented in the original proposal, we predicted that Dkk proteins and Argos both share the colipase fold – although neither has been characterized structurally. If we can understand this structure, and also understand how (in the case of Argos) it is used for interacting with ErbB ligands, we expect to be able to use hDkk proteins as immunologically tolerated 'scaffolds' that will bind ErbB ligands. Such engineered proteins could be developed into valuable therapeutic agents.

To achieve this goal, we need detailed structural information for Argos itself, a structure of Argos in complex with an ErbB ligand, and a structure of an hDkk protein. We described well-diffracting crystals of Argos alone in last year's report, details of which are summarized in Figures 1 and 2. Generating a form of Argos that behaves well enough to express and purify to high levels, and to crystallize, required significant protein engineering, guided largely by a combined genetic and biochemical analysis of Argos that we published in *J. Biol. Chem.* in 2006 [14]. These studies directed us to a 217-amino acid variant of Argos (the wild-type protein has

444 amino acids), which crystallizes readily from 15% PEG 3400, 0.2M $(NH_4)_2SO_4$, 0.1 M KCl, 0.1M Acetate pH 4.6 (Figure 1). The crystals diffract to better than 2.5Å resolution, and have space group C2 with a = 112Å, b = 62Å, c = 73Å and β = 101°.

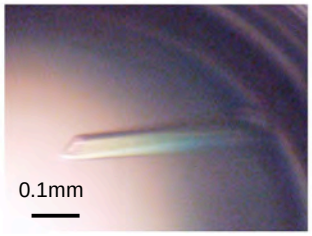


Figure 1
Example of a single crystal of $Argos_{217}$ grown as described in the text.

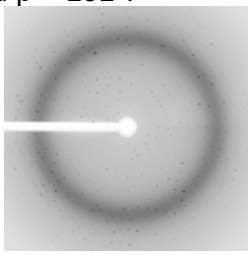
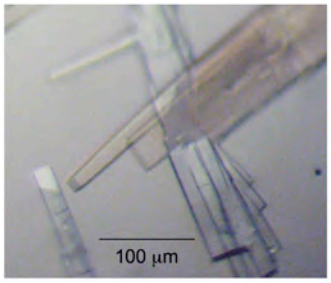


Figure 2
Representative image from a single $Argos_{217}$ crystal taken at APS beamline 23IDin, with 0.5° oscillation angle and a 3 second exposure. This crystal was grown in the presence of 7% glycerol, and frozen directly from the drop.

Solving the Argos structure from these crystals has been very challenging. Although we have an excellent complete native dataset for the unliganded Argos crystals, we have not yet succeeded in obtaining the required phase information to solve the structure. Significant efforts to generate selenomethionine-containing protein have failed. The alternative approach of soaking in heavy atoms for multiple isomorphous replacement has not generated a useful heavy atom derivative, largely because stabilizing the Argos crystals to allow reaction with mercury, platinum, etc compounds has not been possible.

However, we have recently made dramatic progress with crystals of an Argos:ligand complex. In the past year, we succeeded in generating preparations of Spitz, the primary EGF-like ligand in Drosophila. We use proteolysis to cleave the EGF-like domain of Spitz from an engineered variant of the full-length secreted Spitz molecule (produced in Sf9 cells). The resulting ligand crystallizes well – both alone and in complex with $Argos_{217}$.



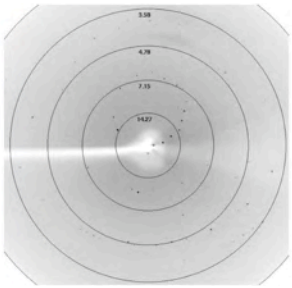


Figure 3

Crystals of an $Argos_{217}$/$Spitz_{EGF}$ complex (left), grown at 21°C from 4% PEG 20K, 100mM Hepes, pH8.0. These crystals diffract to better than 2.5Å resolution on our home source. The image at right shows diffraction of a $KPtCl_4$ derivative on our home source. See text

Our recent crystals of the $Argos_{217}$/$Spitz_{EGF}$ complex diffract sufficiently well for us to have collected a complete dataset to 2.5Å resolution using our home X-ray source. The crystals are of space group P1, with a = 50.4Å, b=52.1Å, and c=69.9Å, α=84.7°, β=75.1° and γ=77.1°. These crystals are much more robust than the $Argos_{217}$-alone crystals, allowing us to soak them in a series of heavy atom solutions. Most recently, we have obtained a clear platinum derivative of the $Argos_{217}$/$Spitz_{EGF}$ crystals, with one clear Pt site that refines well using both anomalous and isomorphous differences, in addition to several other potential sites. A preliminarily calculated SIR/AS map calculated using phases generated from this derivative looks quite promising – although additional phase information is required in order to generate a clearly interpretable map. At the time of writing, we are preparing for a synchrotron trip (at the Advanced Photon Source in Chicago), where it should be possible to measure anomalous differences more accurately for this derivative, and/or to complete a MAD experiment (multiwavelength anomalous dispersion). We are quite confident that these approaches, using

this derivative plus other candidates, will provide interpretable electron density in the very near future, so that we can visualize the high-resolution structure of an Argos/Spitz complex. We have also crystallized the Spitz EGF-like domain alone.

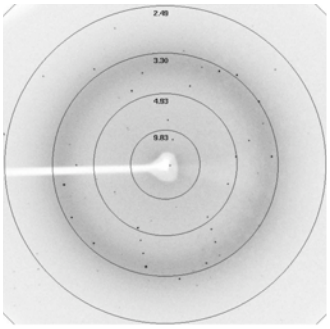


Figure 4
Diffraction of $Spitz_{EGF}$-alone crystals. The crystals diffract to better than 2.5Å using our home X-ray source. They have space group C2, with a=59.8Å, b=36.4Å, c=25.5Å, and $\alpha=\gamma=90^{\circ}$, $\beta=104.1^{\circ}$.

We have collected complete data on our $Spitz_{EGF}$-alone crystals using our home source, providing a complete native dataset and a potential derivative dataset from a crystal soaked in sodium iodide. We are currently analyzing these data for a potential preliminary solution.

We are optimistic that these advances will allow us to determine the crystal structures of free $Spitz_{EGF}$ and its complex with $Argos_{217}$ in the very near future. We should then be able to determine the structure of $Argos_{217}$ alone (from the data mentioned in Figures 1 and 2) using molecular replacement methods. We fully expect to have these structures completed by the summer of 2007.

We have also scaled up production of hDkk1 and hDkk3 from insect cells, as mentioned in the 2006 report. So far, we have not succeeded in growing high quality crystals of these proteins that diffract. We will continue these efforts over the next year, since a direct comparison of Argos and hDkk structures will be very useful for designing strategies that can be employed for conferring Argos-like binding functionality to the hDkk scaffold.

Task 2.
To adapt human Dkk proteins and *Drosophila* Argos to bind and neutralize human ErbB ligands

Since our studies so far indicate that the human Dkk proteins do not bind ErbB ligands, we have focused our attention on engineering or evolving Argos and Dkk proteins to interact with the human EGF-related growth factor. In the original proposal, we described approaches for displaying the basic Argos and Dkk scaffolds as pIII fusions on M13 phage, so that we could select from limited randomized libraries to identify forms of Argos and hDkk that bind human EGF (and other ErbB ligands) with high affinity. As with all phage display projects, one of the first challenges is to display the protein on the phage surface while maintaining function. Since this is usually best achieved with the smallest fragment (or domain) of the protein that retains function, we completed Task 2b first – establishing that $Argos_{217}$ is the minimal Argos fragment that maintains functionality. This was described in the 2006 progress report. In the past year, we have put a great deal of effort into displaying Argos and other relevant proteins as pIII fusions on M13 phage (Task 2a). This has been technically very challenging, possibly because the protein does not fold correctly in this context. This difficulty has resulted in a set-back, but one that we believe we have now overcome by utilizing instead a yeast surface display approach pioneered by Wittrup and colleagues [15]. As described below, we can now display Argos, Spitz, and human EGF on the surface of yeast cells, which will allow us to pursue our screening approaches with this alternative method over the next 12 months.

Task 2a *Establish procedures for displaying the C-terminal cysteine-rich regions of*

hDkk1, hDkk2 and Argos as pIII fusions on M13 phage, and ensure that they are correctly presented by using phage ELISA assays to check binding to positive controls (LRP6 for hDkk1 and hDkk2; Spitz for Argos) (months 1 to 4)

As mentioned above, we were unsuccessful in our efforts to display Argos on the surface of phage M13 – as assessed by a series of attempts to detect Argos directly or associated tags fused to gene III on phage surfaces. We have therefore turned to the yeast-display approach developed by Wittrupp and colleagues [15, 16], which is sold commercially by Invitrogen (as the pYD1 Yeast Display Vector Kit). Proteins of interest are expressed on the surface of *S. cerevisiae*, fused to the Aga2p protein (a component of the **a**-agglutinin receptor). The Aga2p portion of the fusion protein associates through two disulfide bonds with the Aga1p subunit of the **a**-agglutinin receptor, which is itself covalently attached to the outer surface of the yeast cell wall. As a result, the 'test' protein fused to Aga2p (in this case our Argos or hDkk) is covalently associated with the yeast surface. This approach has been validated in studies to evolve regions of the EGFR extracellular region to bind more strongly to anti-EGFR antibodies [17]. Moreover, Jin *et al*. have recently managed to gain a 200,000-fold increase in affinity of an integrin to ICAM-1 with this method [18]. The idea in this case is to perform selection (using the same randomized libraries outlined in the original proposal) using flow cytometry to select yeast clones that are specifically bound by a fluorescently labeled form of the target (human EGF, for example).

As shown in Figure 5, we can readily display either human EGF or the Drosophila Spitz ligand on the yeast surface using this approach. The surface-displayed EGF or Spitz is readily detected using flow cytometry (Fig. 5A) or immunofluorescence (Fig. 5B) with the relevant anti-EGF (or anti-Spitz) antibodies.

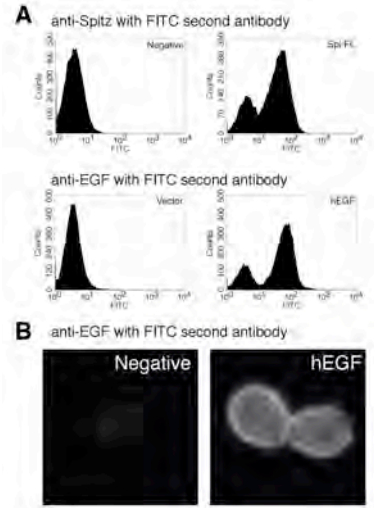


Figure 5
A. Flow cytometry analysis of EBY100 *S. cerevisiae* with anti-Spitz antibody (upper panels) or anti-human EGF antibody (lower panels) detected with FITC-labeled anti-IgG. Cells transfected with derivatives of plasmid pYD1 directing expression of a Spitz/Aga2p chimera (upper right panel) show strong staining with the anti-Spitz antibody. Cells transfected with the EGF-containing pYD1 derivative show strong staining with anti-EGF antibody.

B. The EGF-Aga2p fusion is detected clearly on the surface of yeast cells by immunofluorescence with anti-EGF antibodies.

These data demonstrate that we can readily express EGF-like ligands on the surface of *S. cerevisiae*. EGF and Spitz can also be detected at the cell surface using fluorescently-labeled versions of the *Drosophila* and human EGFR extracellular regions.

We have also shown that the human EGF displayed on the yeast surface is active in binding to its receptor, as is Spitz. Figure 6 shows that yeast expressing hEGF fused to Aga2p bind both to a FITC-conjugated antibody against the histidine tag that it includes and to AlexaFluor-633 labeled sEGFR (the extracellular region of the human EGFR).

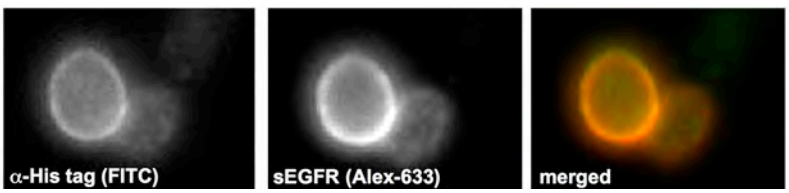


Figure 6
Yeast expressing hEGF-conjugated Aga2p were stained with a FITC-conjugated antibody against the his-tag (left) and labeled sEGFR.

Analysis with flow cytometry also shows that yeast expressing hEGF or Spitz can be detected by this method with an anti-His tag antibody and the isolated extracellular domain of the relevant receptor (Alexa-633 labeled). In Figure 7, cells expressing his-tagged hEGF fused to Aga2p give a range of fluorescence intensities (reflecting heterogeneity in expression level) when treated with a FITC-conjugated antibody against the histidine tag (see Figure 7A). When an Alexa633-labeled form of the extracellular domain from human EGFR is also added, it can be seen in Figure 7B that cells with the highest FITC signal (*i.e.* with the largest numbers of cell-surface his tags) also bind most strongly to sEGFR. These data show both that the surface-displayed hEGF is active in binding to its receptor and that FACS can be used to monitor this system. Figure 7C shows similar results for the Drosophila EGFR ligand Spitz. Cells expressing the largest amount of his-tagged Spitz are also sortable in FACS based on their binding to the Alexa633-labeled extracellular domain of the Drosophila EGF receptor.

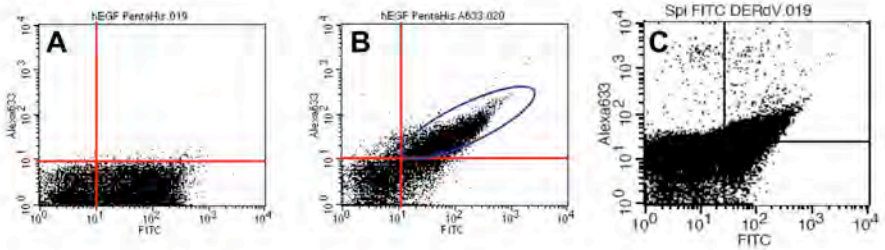


Figure 7
FACS analysis of yeast expressing Aga2p fused to hEGF (A and B) or Spitz (C). See text for details.

Having established that we can express hEGF and Spitz on the surface of EBY100 yeast, we have recently done the same for Argos, and for $Argos_{217}$. Cell surface-displayed $Argos_{217}$ is readily detectable using flow cytometry or immunofluorescence microscopy with an antibody against its histidine tag. Preliminary studies also indicate that it can be detected using Alexa633-labeled Spitz, and these functional studies are now in progress. Once this is completed, we will be in a position to screen libraries of Argos variants using this approach.

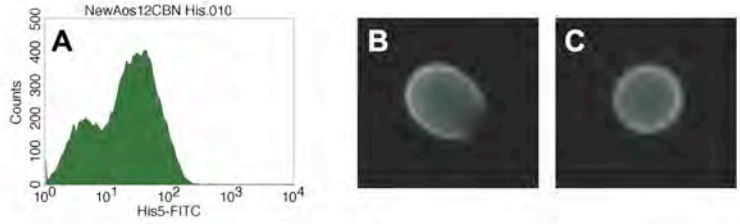


Figure 8
Surface expression of Aga2p-fused $Argos_{217}$ in yeast.
(**A**) the majority of expressing cells are detectable with anti-His antibody. (B and C) show immunofluorescence staining of surface Argos217 with α-His antibody.

These studies establish the experimental basis for the library screening that we proposed in the original application, but using yeast surface display rather than phage display.

Task 2b *Generate a set of nested deletions to determine what are the smallest C-terminal cysteine-rich fragments of hDkk1/2 and Argos that will bind to the positive control targets, and test binding by phage ELISA (months 4 to 8)*

This task was completed using biochemical and genetic approaches for Argos, and was described in the 2006 progress report.

Task 2c *Define conditions under which the observed weak binding of Argos-bearing phage to human EGF can be readily detected - to ensure that some signal can be obtained in f (below), and that this can be 'tuned' by altering stringency.*

This task is now in progress. Having successfully displayed Argos and $Argos_{217}$ on the surface of yeast cells, we are now developing two approaches for selecting Argos mutants. One

approach will employ Alexa633-labeled EGF and Spitz, in combination with flow cytometry. We will be able to use FACS to isolate cells that bind to each ligand, and distinguish between those that bind with higher and lower affinity by altering the stringency of the conditions under which FACS is performed. We are also using biotinylated EGF and Spitz, attached to magnetic beads, to select yeast displaying binding-active Argos variants using biochemical means. This approach was an important pre-screening step prior to FACS in the utilization of this method in studies by other laboratories [18]. We expect to have these approaches fully developed within the next 2-3 months.

Task 2d *Generate mutants for display on M13 to determine which regions of hDkk1/2 and Argos are primarily responsible for defining the specificity of EGF domain recognition, and test these mutants by phage ELISA (months 8 to 12)*

As mentioned in the 2006 report, this task was essentially completed using combined genetic and biochemical approaches, and was published in a collaborative *J. Biol. Chem.* paper in 2006 [14]. The findings of these studies identified two critical parts of the Spitz binding site, which will be focused on when generating randomized $Argos_{217}$ libraries from which to isolate variants that bind strongly to human EGF.

Task 2e *Design and generate phage libraries in which sets of 6 amino acids in a region defined in d above (and structural studies of Argos/Spitz complex) are randomized (months 12 to 16)*

We are in the process of designing and generating libraries in which the predicted loops centered on V146 and S371 in Argos are independently randomized (these were identified as key residues in Spitz binding by Argos). We will begin our screens with these libraries, with the intention of generating more rational, structure-based, libraries when the Argos/Spitz complex crystal structure is completed (and this is imminent, as mentioned above).

Task 2f *Screen phage libraries at a range of different 'stringencies' (defined in c above) to isolate members with improved affinity for immobilized human EGF (months 16 to 24)*

This task is slightly delayed, for the reasons outlined above. However, substituting yeast surface display for phage display, we anticipate initiating this task within the next 2-3 months.

Task 2g *Analyze results of phage display analysis, and design molecules that combine the selected elements in all of the loops randomized. Test these for EGF binding (months 24 to 26).*

We anticipate completing this task in the next year – as planned – but using yeast surface display rather than phage display.

Task 2h *Generate recombinant proteins incorporating the sequences indicated by phage display, and test for binding to human EGF and inhibition of EGF-induced ErbB receptor activation (months 26 to 30)*

We anticipate completing this task in the next year – as planned.

Task 2i *Pursue similar strategies for generating molecules capable of binding to other ErbB ligands (months 30-36 and beyond)*

We anticipate beginning this task in the next year – as planned.

Task 2j *Develop strategies with collaborators for further testing of human Argos equivalents in more physiological settings.*

We hope to initiate these efforts in the next year – as planned.

KEY RESEARCH ACCOMPLISHMENTS

- Crystallized an $Argos_{217}$/Spitz complex, and collected complete native data
- Crystallized Spitz alone, and collected diffraction data
- Using a platinum derivative, generated preliminary experimental phase information (this should be completed following an imminent synchrotron trip)
- Determined that we cannot display function Argos on the surface of phage M13, requiring a change in experimental strategy
- To replace the phage display, we have established the yeast surface display system in the laboratory, and have shown that we can display active EGF, Spitz, and Argos on the surface of *S. cerevisiae*

REPORTABLE OUTCOMES

1. Results presented in invited talks at a meeting of the Ludwig Institute for Cancer Research (May, 2006), a meeting on Protein Phosphorylation and Cell Signaling at the Salk Institute (August 2006), NIDDK (December 2006), and Vanderbilt University (January 2007).

2. A paper was published describing identification of mutated forms of Argos that no longer bind Spitz:
   D. Alvarado, T.A. Evans, R. Sharma, M.A. Lemmon, and J.B. Duffy.
   "*Argos mutants define an affinity-based threshold for Spitz inhibition in vivo*",
   J. Biol. Chem. **281,** 28993-29001.
   Additional manuscripts are in preparation.

CONCLUSIONS to date

Our central aim is to develop novel proteins with the ability to bind to and inactivate the growth factors that activate receptors from the human EGF receptor family. This aim follows from our discovery that Argos, an EGFR inhibitor in *Drosophila melanogaster*, functions by sequestering the activating ligands in Drosophila. We had identified human Dickkopf (Dkk) proteins as potential Argos equivalents, based on their predicted pattern of disulfide bonding (and copurification with neuregulin in one reported study), and hypothesize that they will share a similar overall structure to Argos. In 2005-2006, we established that Dkk proteins do not bind human ErbB ligands, indicating that the simplest possible route to discovering a human 'anti-EGF' will not work. Therefore, in the past year, we turned to the alternative (and most likely) strategy described in detail in the original proposal - to use Dkk proteins and Argos as structural 'scaffolds' that will be subjected to selection through phage display for molecules that can neutralize human EGF-related molecules.
We previously identified the smallest fragment of Argos ($Argos_{217}$) that is capable of neutralizing Drosophila EGFR ligands. We expect within the next few months to have a high resolution structure of this protein in complex with its natural ligand Spitz, plus structures of $Argos_{217}$ and Spitz alone. These structures will be very important not only for understanding the basis for Argos action, but also in our efforts to design Argos mimics that will neutralize human ligands. Using these results, plus our recently-published identification (by mutagenesis) of two key loops in Argos that are critically involved in interactions with EGF ligands, we are now in a position to make rationally targeted randomized libraries from which to select Argos variants that might bind human EGF (our aim for therapeutic design). Technically, the past year has shown us that our plans to use phage display for screening these libraries is unlikely to be successful. We have therefore turned to yeast surface-display technology, which looks very promising based on our studies to date. Our next steps are to generate the recently-designed libraries, to display them on the yeast surface, and to screen for interactors with human EGFR ligands. The imminent Argos/Spitz complex structure will also inform more rational library design.

So What ?

Argos is an inhibitor of EGF receptor signaling used in Nature to controlling signaling through this receptor in *D. melanogaster*. Aberrant signaling through the EGF receptor and its relatives is well known to play an important role in breast and other cancers. We propose to follow Nature's example, and design (or discover) a molecule that can function like Argos in breast cancer patients where EGF receptor family signaling is over-active. We have made significant strides in understanding the way in which Argos binds to and neutralizes the growth factors that activate the EGF receptor, and have determined the minimum protein required for this – as well as identifying key parts of its interaction site. Work over the past year has brought us to the verge of determining a high-resolution crystal structure of the Argos/Spitz complex. We have also made several important technical advances (and course corrections) that have brought us to a stage where – with a year of the grant to go – we are now ready to screen from rationally limited libraries for Argos variants that will bind human EGF-like ligands, and that will represent first-model potential anti-cancer therapeutics. Our studies are on course, and we anticipate having molecules in the next 12 months that will have the ability to neutralize human growth factors (and thus be starting points for development as therapeutics).

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